Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L2	4	indole-3-glyoxamide	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:50
L3	12	indole adj2 glyoxamide	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:50
L4	3	indoleglyoxamide	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:50
L5	32	glyoxamides	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:52
L6	36356	ischemia or reperfusion or ischemic	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:51
L7	1	l5 and l6	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:51
L8	38	l2 or l3 or l4 or l5	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:53
L9	19	l8 and (heart or kidney or pancreas or liver)	US-PGPUB; USPAT	OR	OFF	2005/07/22 13:53

Ischemia/reperfusion (I/R) results in tissue injury in a number of organs, including the **heart**, brain, **kidney**, and the gastrointestinal tract, with important implications for patient morbidity and mortality (1). Although several mechanisms have been proposed to explain the pathogenesis of I/R injury, most of the attention has focused on the role of reactive oxygen species (ROS): superoxide radical ($O_2^{\cdot}$), hydroxyl radical (*OH), hydrogen peroxide (H_2O_2) and inflammatory leukocytes (2). During **ischemia**, the interruption of blood supply and the lack of oxygen lead to anaerobic metabolism, with a loss of energy substrates and the accumulation of hypoxanthine within the ischemic cells. The depletion of energy substrates affects the membrane ionic ATPase pumps, tending to result in the accumulation of calcium, sodium and water in the cells, which in turn causes them to swell. **Reperfusion** stimulates the conversion of hypoxanthine and xanthine to uric acid and the excessive production of ROS. This can lead to lipid peroxidation (3) and protein oxidation of cell membranes (4).
Inflammatory leukocytes, especially neutrophils, generate and release cytotoxic compounds, while adhering to vascular endothelium and infiltrating the tissue (5). An oxidative or respiratory burst in neutrophils during **reperfusion** results in the production of ROS. The extracellular release of cytotoxic products from neutrophils initiates lipid peroxidation and protein oxidation that cause tissue injury.
Renal injury can also occur in neutrophil-independent pathways, as seen in neutropenic patients who develop acute renal failure, indicating that neutrophils are a factor, but not the only factor, that contributes to acute renal failure (6).
Many studies have shown the protective effects of antioxidants in I/R injury in the **heart**, the liver, the **intestine** and the **kidney**, suggesting an important role of ROS in pathogenesis (7). Quercetin (3, 5, 7, 3'and 4'-pentahydroxy flavonol) is a powerful antioxidant with metal-ion, i.e. Fe and Cu, binding properties and radical scavenging abilities (8, 9). Prevention of Fe- and Cu-mediated hydroxyl radical formation, combined with a lower peroxide generating power, could make quercetin much more effective in protection against oxidative damage. It has also been reported that quercetin has anti-inflammatory, anti-ischemic and anti-peroxidative properties (10-12).
This study was designed to identify the role of quercetin in I/R-induced oxidative stress in the **kidney** of rats.

Methods

Animals

Sprague-Dawley rats weighing 200-250 g were supplied from The Center of Medical and Surgical Investigation of Osmangazi University, Eskis ¸ehir. The rats were fed with a standard rat chow (Oguzlar Yem, Eskisehir, Turkey) and allowed to freely drink water. The experimental procedures were conducted after obtaining permission from local ethical committees. The rats were anesthetized with thiopental (50 mg/kg, intraperitoneally) and placed on a temperature-regulated table (37°C ± 0.5°C) to maintain body temperature. The abdominal region was shaved with a safety razor and sterilized with povidone iodine solution. A midline incision was made and the right **kidney** was harvested. A non-traumatic vascular clamp was applied to the left renal pedicle. The control group (n=5) underwent identical surgical treatment, including isolation of the left renal pedicle; however, the pedicle occlusion was not performed. **Ischemia** was applied for 45 min to the I/R group (n=5) followed by 60 min of **reperfusion**. The rats in the I/R+Quercetin (I/R+Q) group (n=5) were pretreated intraperitoneally with a quercetin suspension (50 mg/kg) in physiological saline 60 min before the **ischemia** induction. The control group and the I/R group received a comparable volume of vehicle, physiological saline. At the end of each experimental procedure, the left kidneys were removed and kept frozen at -20°C until analysis.

Biochemical assays

The chemicals were purchased from Sigma Chemical Co (St Louis, MO). A portion of each left **kidney** was homogenized for all assays except myeloperoxidase (MPO) assay. Homogenization was performed in 1:10 (w/v) 0.1 M potassium phosphate buffer (pH=7.4) with an Ultra Turrax homogenizer (IKA T18 basic, Wilmington NC, USA). The homogenates were centrifuged at 5000 rpm, +4°C for 10 min. The supernatants were removed and used for further analysis. For MPO assay, renal tissue was first homogenized in 1:10 (w/v) 50 mM potassium phosphate buffer (pH=7.4). After centrifugation at 15000 rpm, + 4°C for 10 min, the pellet was re-homogenized in an equal volume of 50 mM potassium phosphate buffer (pH=6.0) containing 0.5% hexadecyl-trimethylammonium
bromide (HETAB) and 10 mM ethylenediaminetetraacetic acid (EDTA). This homogenate was subjected to MPO assay.
Thiobarbituric acid reactive substances (TBARS) levels were measured by the modified method of Ohkawa et al (13). Protein carbonyl contents were determined using a colorimetric assay measuring the protein carbonyl content after the reaction of the tissue homogenates supernatant with dinitrophenil hydrazine, as described by Levine et al (14). Tumor necrosis factor alpha (TNF-*) levels were determined using a commercially available mouse ELISA kit (MedSystems Diagnostics GmbH, Vienna, Austria). Reduced glutathione (GSH) levels and catalase (CAT) (H_2O_2:H_2O oxidoreductase, E.C.1.11.1.6) activities were measured by Beutler's methods (15, 16). Superoxide dismutase (SOD) (E.C.1.15.1.1) activities were determined by the method of Winterbourn et al (17). MPO activities were measured in the homogenate according to Suzuki et al (18). Protein levels were determined by the Biuret Method. Results are expressed as nmol/mg protein for TBARS levels, protein carbonyl contents and

GSH, as pmol/mg protein for TNF-*
levels, and as U/mg protein for SOD, CAT and MPO activities.

Statistical analysis

Results are expressed as means ± standard error of mean. For statistical analysis, the non-parametrical Mann-Whitney U was used. A p-value of less than 0.05 was considered significant.

Results

As in Figures 1, 2, 3 and 4, TBARS levels as an index of lipid peroxidation, protein carbonyl content as an index of protein oxidation, TNF-* levels as critical early mediators of organ injury and MPO activity as an index of infiltration of neutrophils in the renal tissue were significantly higher in the I/R group than those of the control group ($p<0.05$, $p<0.01$, $p<0.01$ and $p<0.01$, respectively). However, in the I/R+Q group TBARS, protein carbonyl content, TNF-* levels and MPO activity were significantly lower than in the I/R group ($p<0.05$, $p<0.05$, $p<0.01$ and $p<0.01$, respectively). In addition, pretreatment with quercetin significantly decreased the renal TNF-* levels and MPO activities in the I/R+Q group when compared to the control group ($p<0.01$). GSH levels, CAT, and SOD activities significantly decreased in the I/R group when compared to the control group ($p<0.01$, $p<0.05$, $p<0.05$, respectively). Quercetin treatment increased GSH levels and these enzyme activities in comparison with the I/R group ($p<0.05$, $p<0.01$ and $p<0.05$, respectively) (Figs. 5-7).

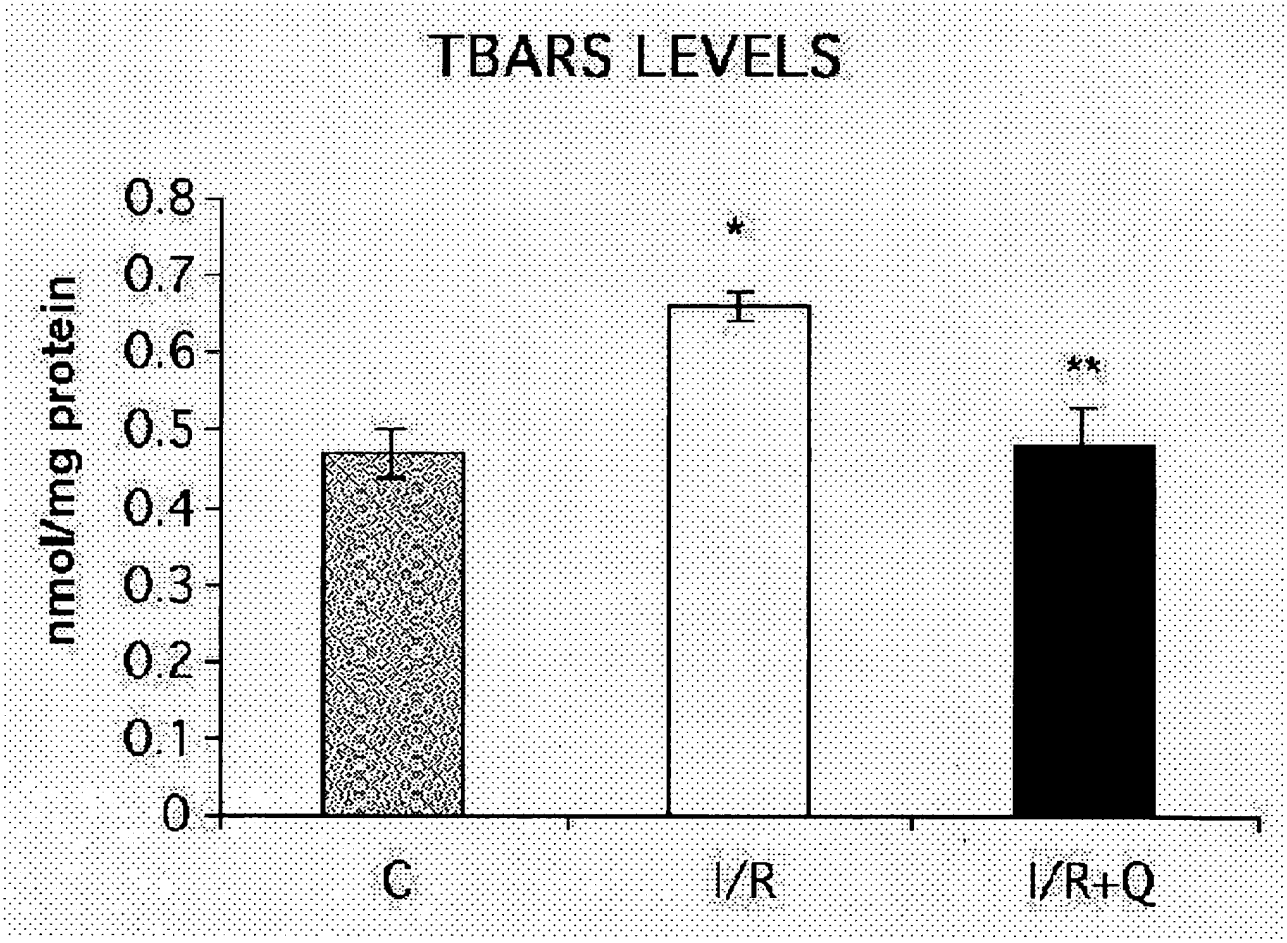

Fig. 1 - Renal tissue TBARS levels in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with quercetin (50 mg/kg) (I/R+Q).
*** p<0.05 vs. control group**
**** p<0.05 vs. I/R group.**

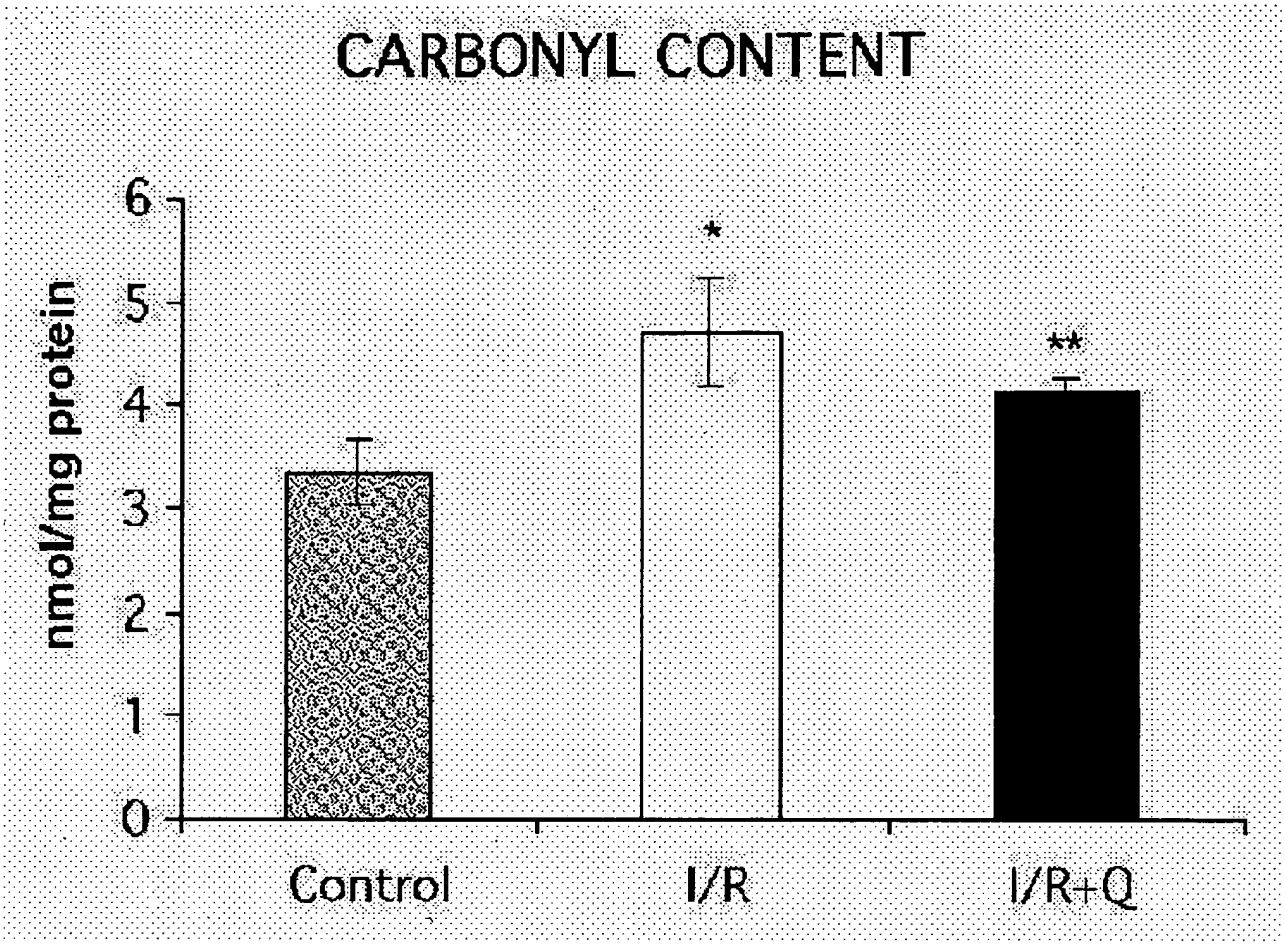

Fig. 2 - Renal tissue protein carbonyl content levels in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with quercetin (50 mg/kg) (I/R+Q).
*** p<0.01 vs. control group**
**** p<0.05 vs. I/R group.**

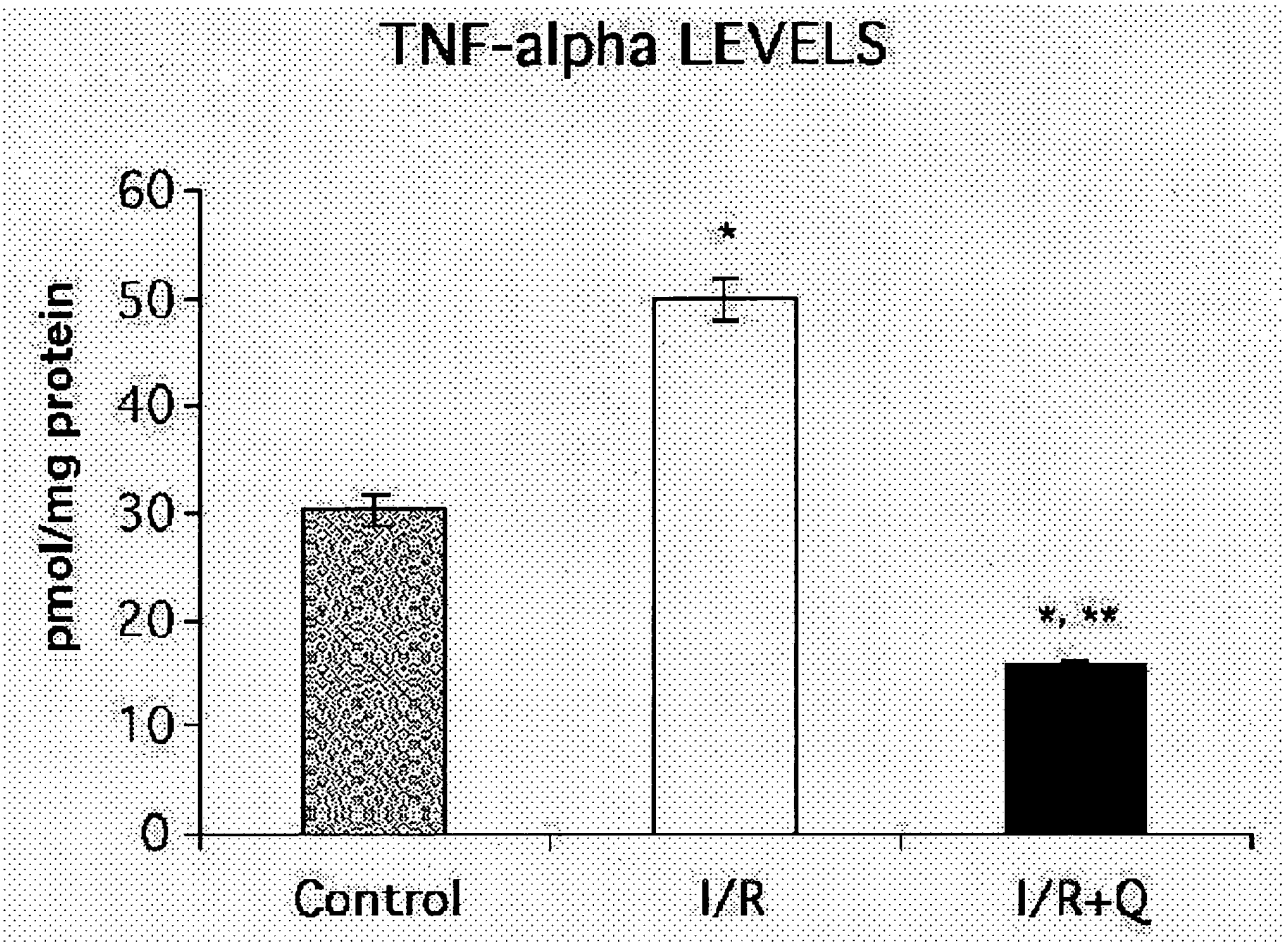

Fig. 3 - Renal tissue TNF-* levels in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with quercetin (50 mg/kg) (I/R+Q).
*** p<0.01 vs. control group**
**** p<0.01 vs. I/R group.**

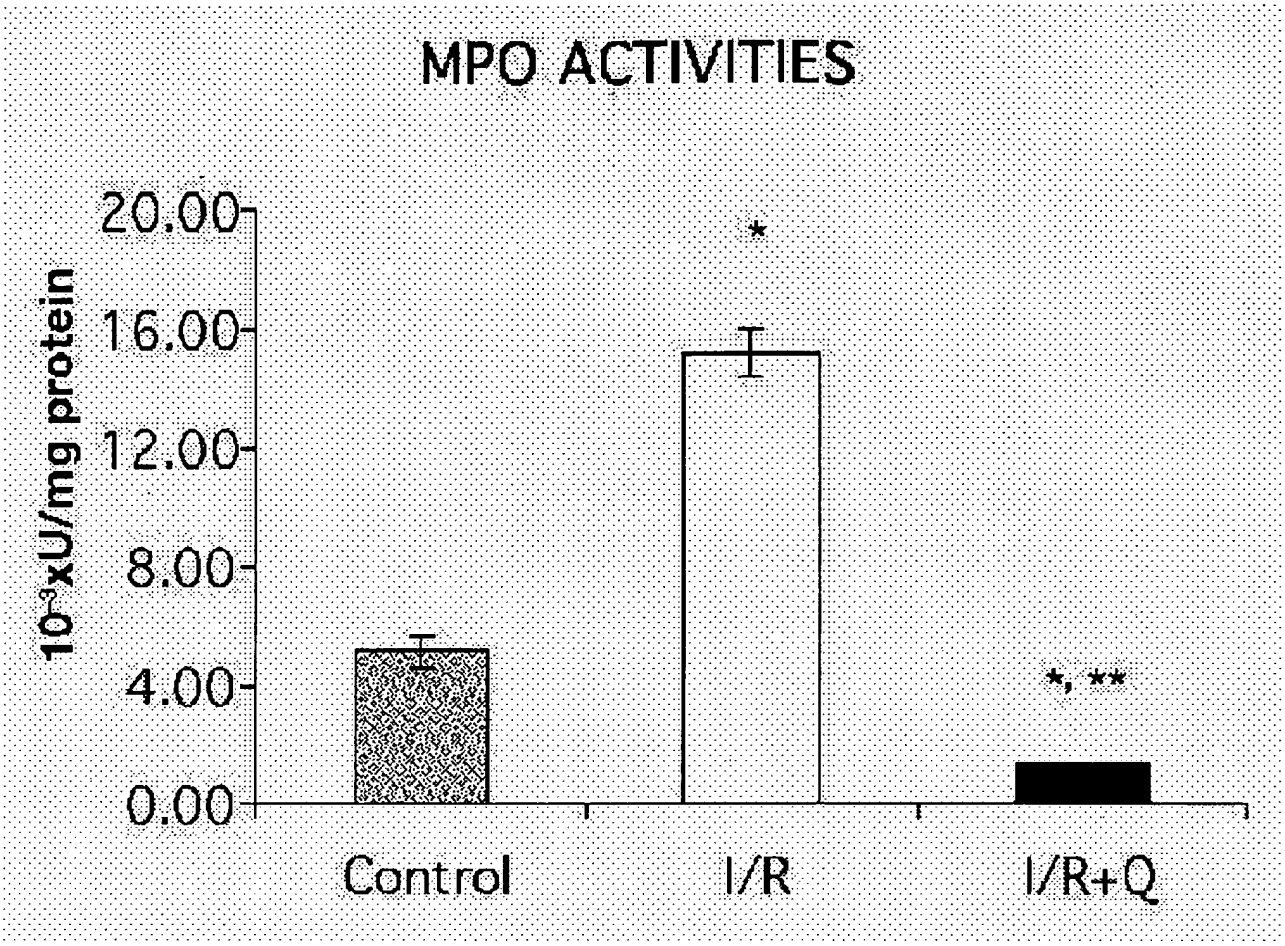

Fig. 4 - Renal tissue MPO activities in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with quercetin (50 mg/kg) (I/R+Q).
*** p<0.01 vs. control group**
**** p<0.01 vs. I/R group.**

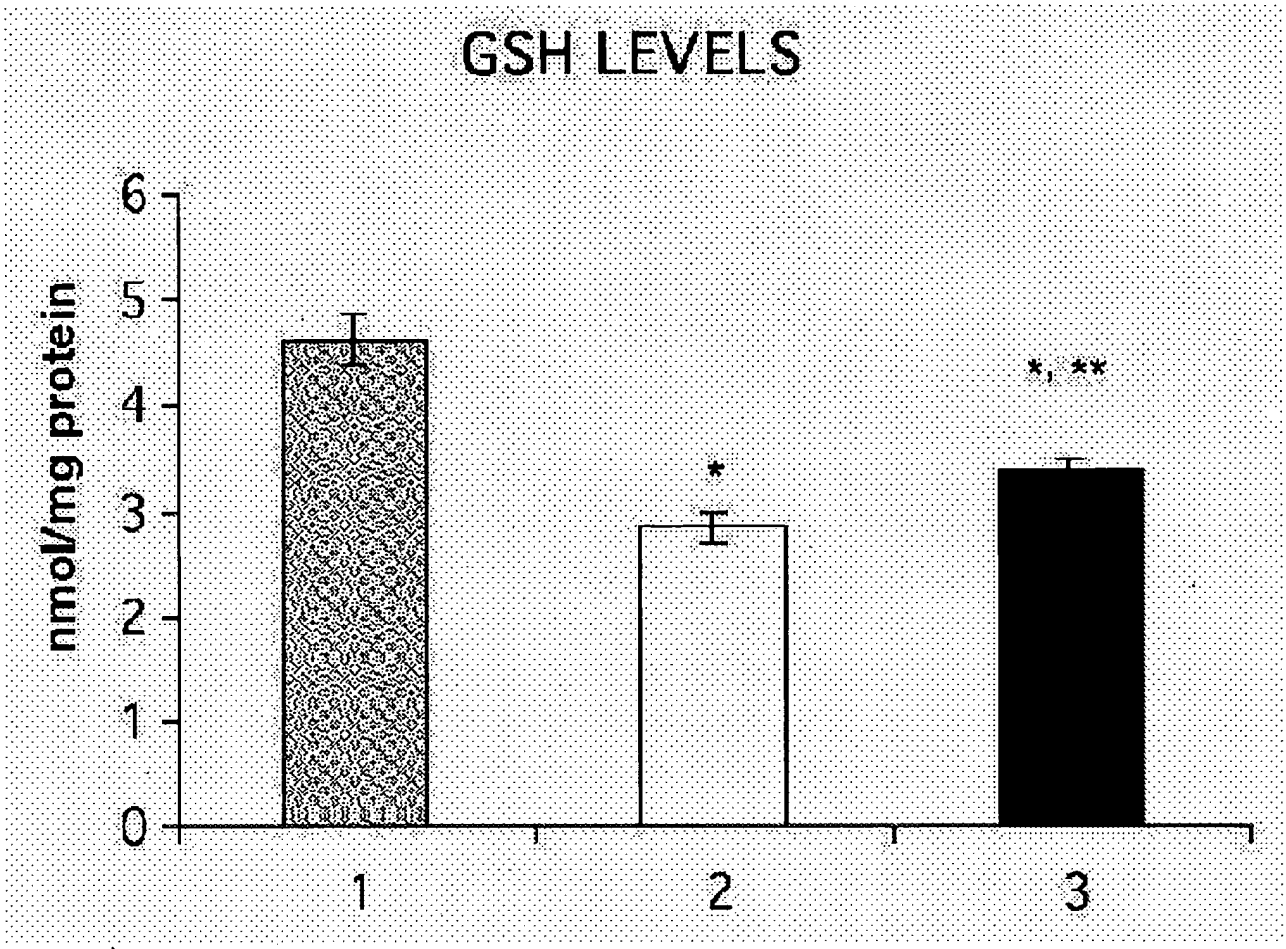

Fig. 5 - Renal tissue GSH levels in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with quercetin (50 mg/kg) (I/R+Q).
*** p<0.01 vs. control group**
**** p<0.05 vs. I/R group.**

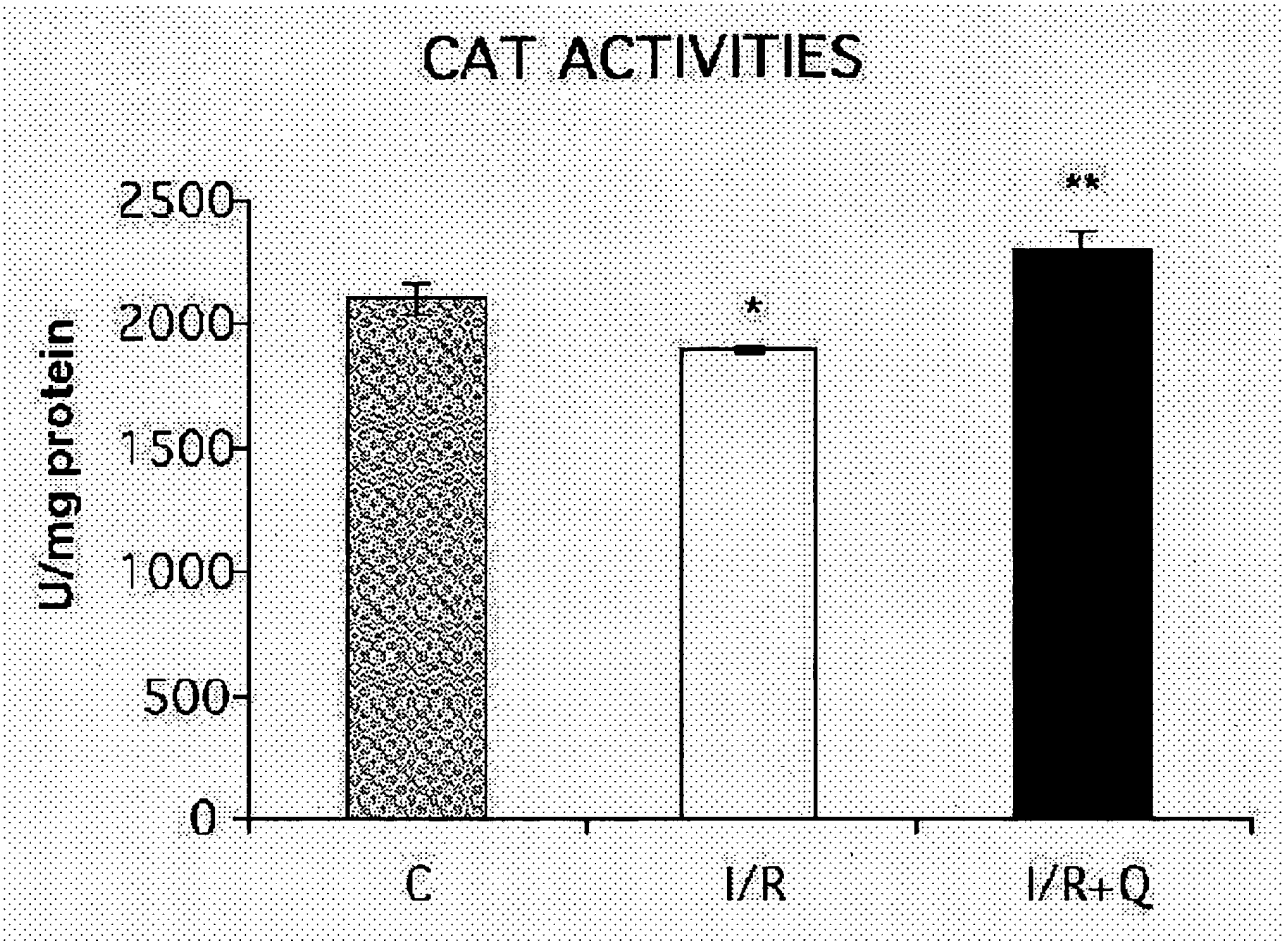

Fig. 6 - Renal tissue CAT activities in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with quercetin (50 mg/kg) (I/R+Q).
*** p<0.05 vs. control group**
**** p<0.01 vs. I/R group.**

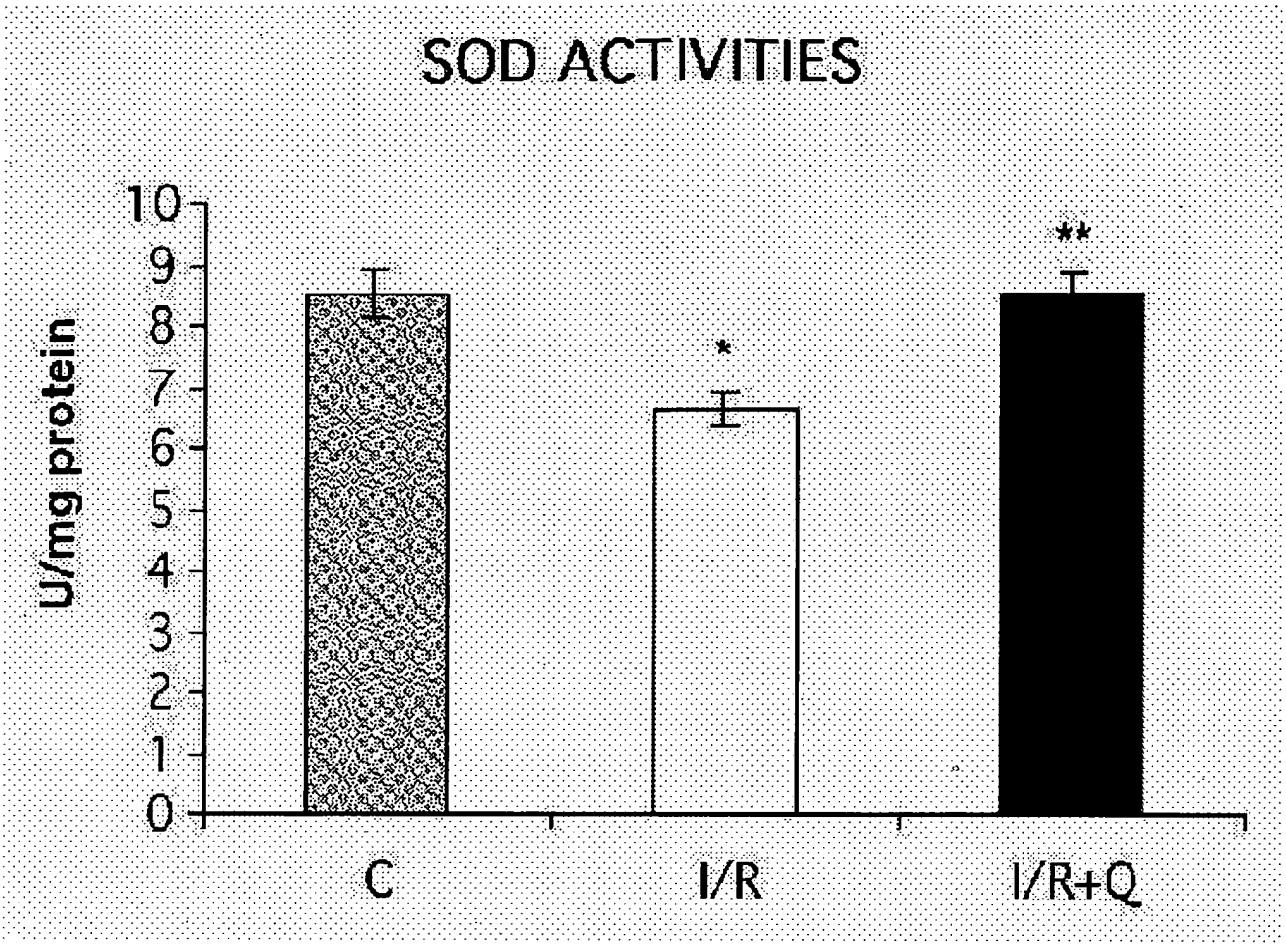

Fig. 7 - Renal tissue SOD activities in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with quercetin (50 mg/kg) (I/R+Q).
*** p<0.05 vs. control group**
**** p<0.05 vs. I/R group.**

Discussion

Our data demonstrated that quercetin plays an important role in the attenuation of I/R-induced renal injury by decreasing TBARS, protein carbonyl, TNF-* levels and MPO activities and by increasing GSH, SOD and CAT activities.
The hypoxanthine oxidation that arises from the degradation of adenosine triphosphate to xanthine and uric acid is catalyzed by the enzyme xanthine oxidase during the **ischemia** followed by **reperfusion**. $O_2^{\cdot}$ ensues from this reaction as a by-product. $O_2^{\cdot}$ and H_2O_2 are converted to $^{\cdot}OH$ via the Fenton and Haber-Weis reactions. $^{\cdot}OH$ initiates lipid peroxidation and protein oxidation of the cell membranes. In addition, the oxidative burst results in the production of ROS. TNF-* is a critical early mediator of organ injury. Donnahoo et al (19) have shown that I/R induced renal TNF expression.
Quercetin is a scavenger of $O_2^{\cdot}$ and $^{\cdot}OH$ (9, 20, 21). This flavonoid was shown to be effective in attenuating the expression of inflammatory chemokines in rat kidneys subjected to I/R (22, 23). In addition, it has also been reported that quercetin inhibits xanthine oxidase activity (24, 25).
This study demonstrated the increasing levels of TBARS, protein carbonyl and TNF-* in the I/R group. Quercetin treatment significantly decreased levels of TBARS, protein carbonyl and TNF-* when compared to the I/R group. In addition, quercetin increased GSH levels when compared to the I/R group. In a previous study, we reported that quercetin treatment decreased malondialdehyde levels in the liver and skin of rats exposed to UVA light, which can induce the production of ROS, and that it increased GSH levels (9, 26).
Superoxide radicals formed by I/R injury are converted into H_2O_2, either spontaneously (in pH 4.8) or by dismutation with the SOD enzyme (especially, in neutral and alkaline pH). H_2O_2 is then converted to H_2O by either CAT or glutathione peroxidase. It has been reported that SOD activity was reduced after I/R injury (27, 28). Dobashi et al (27) also demonstrated mRNA levels of CAT significantly decreased after I/R. In our study SOD and CAT activities were found to be significantly decreased in the I/R group when compared to the control group. The decrease in renal SOD and CAT activities is probably the result of the inactivation by

ROS produced by I/R. Quercetin treatment increased levels of these enzymes in comparison with the I/R group. The increase in the SOD and CAT activities is possibly due to the scavenging of ROS, i.e. $O_2^{\cdot}$ and $^{\cdot}OH$ by quercetin (11, 20).

Circulating neutrophils play a critical role in the pathogenesis of tissue injury provoked by I/R. Neutrophils can contribute to I/R injury by various mechanisms, i.e. production of ROS or proteases and lipid mediators, leukotrienes and platelet-activating factors that affect vascular tone and permeability, exacerbating tissue **ischemia** (29). TNF-* stimulates neutrophils to produce $O_2^{\cdot}$ and H_2O_2 and other toxic metabolites. These ROS released by activated neutrophils and endothelial cells have been implicated in I/R injury induced by neutrophil accumulation. In addition, Ysebaert et al suggested that MPO activity in the early post-**ischemia** period probably reflects not only neutrophils but also the other adhering inflammatory cells such as monocytes/macrophages (30) We measured MPO activity as a marker of inflammatory cell activation. Some authors reported that the exposure of renal tissue to I/R caused a significant increase in MPO activity (30, 31). According to our results, quercetin, which has anti-inflammatory properties, prevented the increase in MPO activity and, therefore, protected renal tissue from the deleterious effects of activated inflammatory cells. These results agree with other reports explaining the anti-inflammatory activity of quercetin (32).

Finally, these results indicate that the renoprotective effects of quercetin in the renal injury induced by I/R could be related to its antioxidant properties, which reduce the lipid peroxidation and protein carbonyl compounds, and increase GSH levels, SOD activity, CAT activity, and anti-inflammatory properties. Therefore, quercetin can have a role in therapeutic regimens that are both immunosuppressive and renoprotective.

Address for correspondence: Ahmet Kahraman, Ph.D. - *Department of Biochemistry, The School of Medicine Kocatepe University Afyon-03200, Turkey ahmetkah@aku.edu.tr*

References

1. Kelly KJ, Jr. Williams WW, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos J-C, Bonventre JV. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. J Clin Invest 1996; 97: 1056-63. PMID: 12761255
2. Granger DN, Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. Annu Rev Physiol 1995; 57: 312-32. PMID: 7778871
3. Hauet T, Michel G, Vandewalle A. To what extent can limiting cold **ischemia/reperfusion** injury prevent delayed graft function? Nephrol Dial Transplant 2001; 16: 1982-5. PMID: 11572883
4. Stadtman ER, Berlett BS. Free-radical-mediated modification of proteins. In Wallace KB, ed. Free radical toxicology. Taylor&Francis, 1997; 71-87. PMID: 12162447
5. Singbartl K, Ley K. Protection from **ischemia-reperfusion** induced severe acute renal failure by blocking E-selectin. Crit Care Med 2000; 28: 2507-14. PMID: 10921586
6. Heinzelmann M, Mercer-Jones MA, Passmore J. Neutrophils and renal failure. Am J **Kidney** Dis 1999; 34: 384-9. PMID: 10430993
7. Jaeschke H, Mitchell JR. Use of isolated perfused organs in hypoxia and **ischemia/reperfusion** oxidant stress. Methods Enzymol 1990; 186: 752-9. PMID: 2233332
8. Szeto YT, Benzie IFF. Effects of dietary antioxidants on human DNA ex vivo. Free Radic Res 2002; 36: 113-8. PMID: 11999698
9. Inal ME, Kahraman A. The protective effect of flavonol quercetin against ultraviolet A induced oxidative stress in rats. Toxicology 2000; 154: 21-9. PMID: 12355559
10. Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol 1995; 33: 1061-80. PMID: 8847003
11. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 1988; 37: 837-41 PMID: 2830882
12. Terao J, Piskula M, Yao Q. Protective effect of epicatechin, epicatechin gallate and quercetin on lipid peroxidation in phospolipid bilayers. Arch Biochem Biophys 1994; 308: 278-84. PMID: 8311465
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidase in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8. PMID: 36810
14. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A, Ahn BW, Shaltiel S, Stadman ER. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990; 186: 464-78. PMID: 1978225
15. Beutler E, Robson MJ, Buttenwieser E. The glutathione instability of drug-sensititive red cells. J Lab Clin Med 1957; 49: 84-95. PMID: 12707220
16. Beutler E, ed. Red cell metabolism, a manual of biochemical methods. New York: Grune & Stratton, Inc 1973; 76-85. PMID: 12649162
17. Winterbourn CC, Hawkins RE, Brian M, Correll RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1957; 49: 84-95. PMID: 803541
18. Suzuki K, Ota H, Sasagawa S, Sakatani T, Fujikura T. Assay method for myloperoxidase in human polymorphonuclear leukocytes. Anal Biochem 1983; 132: 345-52. PMID: 6312841

19. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. Early **kidney** TNF-* expression mediates neutrophil infiltration and injury after renal **ischemia-reperfusion**. Am J Physiol 1999; 46: R922-9. PMID: 10484513
20. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 1987; 26: 2489-91. PMID: 12758013
21. Bors W, Heller W, Michel C, Saran M. Flavonoid as antioxidants: Determination of radical-scavenging efficiencies. Methods Enzymol 1990; 186: 143-55. PMID: 2244490
22. Jones EA, Shoskes DA. The effect of mycophenolate mofetil and polyphenolic bioflavanoids on renal **ischemia reperfusion** injury and repair. J Urol 2000; 163: 999-1004. PMID: 10688038
23. Shoskes DA. Effect of quercetin and curcumin on ischemic renal injury: a new class of renoprotective agents. Transplantation 1998; 66: 147-52. PMID: 11543813
24. Sanhueza J, Valdes J, Campos R, Garido A, Valezuela A. Changes in the xanthine dehydrogenase/xanthine oxidase ratio in the rat **kidney** subjected to **ischemia-reperfusion** stress: preventative affect of some flavonoids. Res Commun Chem Pathol Pharmacol 1992; 78: 211-8. PMID: 1475527
25. Nagao A, Seki M, Kobayashi H. Inhibition of xanthine oxidase by flavonoids. Biosci Biotechnol Biochem 1999; 63: 1787-90. PMID: 10671036
26. Inal ME, Kahraman A, Koken T. Beneficial effects of quercetin on oxidative stress induced by ultraviolet A. Clin Exp Dermatol 2001; 26: 536-9. PMID: 12355559
27. Dobashi K, Ghosh B, Orak JK, Singh I, Singh AK. **Kidney ischemia-reperfusion**: Modulation of antioxidant defenses. Mol Cell Biochem 2000; 205: 1-11. PMID: 10821417
28. Yüceyar S, Gümüs ¸tas ¸ K, Erturk S, Hamzaog (breve)lu IH, Uygun N, Ayaz M, Cengiz A, Kafadar Y. The role of oxygen free radicals in acute renal failure complicating obstructive jaundice: an experimental study. HPB Surg 1998; 10: 378-93. PMID: 12764668
29. Colletti LM, Remick DG, Burtch GD, Kunkel SL, Sterieter RM, Campbell DA Jr. Role of tumor necrosis factor-* in the pathologic alterations after hepatic **ischemia-reperfusion** injury in the rat. J Clin Invest 1990; 85: 1936-43. PMID: 2161433
30. Ysebaert DK, De Greef KE, Vercauteren SR, Ghielli M, Verpooten GA, Eyskens EJ, De Broe ME. Identification and kinetics of leukocytes after severe **ischemia/reperfusion** renal injury. Nephrol Dial Transplant 2000; 15: 1562-74. PMID: 11007823
31. Tsuruma T, Yagihashi A, Watanabe N, Yajima T, Kameshima H, Araya J, Hirata K. Heat-shock protein-73 protects against small intestinal warm **ischemia-reperfusion** injury in the rat. Surgery 1999; 125: 385-95. PMID: 10216529
32. Stavric B. Quercetin in our diet: from potent mutagen to probable anticarcinogen. Clin Biochem 1994: 27: 245-8. PMID: 8001284

Received: *November 14, 2002* ***Revised:*** *March 12, 2003* ***Accepted:*** *March 27, 2003*